Date of Approval: May 2, 2016

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-439

INTEPRITY

avilamycin Type A medicated article

Broiler chickens

For the prevention of mortality caused by necrotic enteritis associated with *Clostridium perfringens* in broiler chickens

Sponsored by:

Elanco Animal Health,
A Division of Eli Lilly & Co.

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I. GENERAL INFORMATION

A. File Number

NADA 141-439

B. Sponsor

Elanco Animal Health,
A Division of Eli Lilly & Co.
Lilly Corporate Center
Indianapolis, IN 46285

Drug Labeler Code: 000986

C. Proprietary Name

INTEPRITY

D. Established Name

Avilamycin Type A medicated article

E. Pharmacological Category

Antimicrobial

F. Dosage Form

Type A medicated article

G. Amount of Active Ingredient

45.4 g/lb (100 g/kg)

H. How Supplied

25 kg (55.12 lb) bag

I. Dispensing Status

Veterinary Feed Directive (VFD)

J. Dosage Regimen

Feed at 13.6 to 40.9 grams per ton of Type C medicated feed (15 to 45 ppm) as the sole ration for 21 consecutive days.

K. Route of Administration

Oral

L. Species / Class

Broiler chickens

M. Indication

For the prevention of mortality caused by necrotic enteritis associated with *Clostridium perfringens* in broiler chickens

II. EFFECTIVENESS

A. Dosage Characterization

A study was conducted to evaluate the ability of avilamycin medicated feed to prevent necrotic enteritis-related mortality and intestinal lesions associated with a *Clostridium perfringens* challenge. Inclusion rates of 15, 30, and 45 ppm were chosen based on anecdotal evidence from global use of avilamycin that suggested inclusion rates above 10 ppm in feed were necessary to prevent necrotic enteritis-related mortality.

The study utilized 648 commercial broiler chicks. Medicated feed was administered beginning on Day 0 (day of hatch) or Day 15. The *C. perfringens* challenge was administered on Day 14. Inclusion rates of 15, 30, and 45 ppm administered for 21 days beginning on Day 0, and 45 ppm administered for 6 days beginning on Day 15, all resulted in lower necrotic enteritis-related mortality compared to the non-medicated challenged control group. Based on these results, a minimum inclusion rate of 15 ppm was chosen for the clinical effectiveness studies.

B. Substantial Evidence

Effectiveness of avilamycin for the prevention of mortality due to necrotic enteritis associated with *C. perfringens* was demonstrated using two experimentally-induced infection model studies.

1. Induced Infection Challenge Model Study

a. Title: "Clinical Study: Examination of the Ability of Avilamycin to Control Mortality Related to *Clostridium perfringens* (Necrotic Enteritis) in Flocks of Growing Chickens". Elanco Study Number T4ECA100002; Nutreco Study Number ZB1993E. January 2011 to February 2011.

b. Study Location: Ontario, Canada.

c. Study Design:

(1) Objective: To confirm the effectiveness of avilamycin, administered as a feed medication, for prevention of mortality and lesions associated with *C. perfringens* in growing chickens resulting from an induced outbreak of clostridial enteritis (necrotic enteritis). The study was conducted according to the VICH Good Clinical Practice (GCP) Guideline (VICH GL9; FDA Guidance For Industry #85).

(2) Study Animals: The study enrolled 2,200 day-of-hatch, male Ross 708 strain broiler chicks. The birds were from the same hatch group and breeder flock. The chicks were randomized to study pens on the day of arrival from the hatchery (Day 0). Each pen contained 55 chicks. The chicks were managed, housed, fed, and watered according to standard, indoor poultry management practices. The chicks were feed three

consecutive commercial stage-specific diets: starter feed from hatch to 21 days of age, grower feed from 21 days of age to 30 days of age, and finisher feed from 30 days of age to market weight at 35 days of age.

(3) Treatment Groups: Chicks were randomized to one of four treatments as provided in Table II.1.

Table II.1. Avilamycin Treatment Groups

Treatment Group	Clostridial Challenge	Treatment Regimen	Number Treated
Group 1	No	0 ppm avilamycin, in feed for 23 days	550
Group 2	Yes	0 ppm avilamycin, in feed for 23 days	550
Group 3	Yes	15 ppm avilamycin, in feed for 23 days	550
Group 4	Yes	30 ppm avilamycin, in feed for 23 days	550

(4) Infection Challenge Administration: On Day 14, Groups 2, 3, and 4 were challenged with a *C. perfringens* isolate that induces representative disease in challenged chickens. The isolate was initially collected in November 2001 from a broiler chicken that died during a spontaneous necrotic enteritis outbreak in North America. Following a 6 to 8 hour period of feed withdrawal, the *C. perfringens* challenge was administered in non-medicated starter feed for approximately 16 hours.

(5) Drug Administration: The test article was avilamycin, which was administered via a complete Type C medicated feed. The Type C medicated feed was made from a 10% avilamycin Type A medicated article in the final marketed formulation. Medicated complete feed was provided to Group 3 (15 ppm) and Group 4 (30 ppm) for 23 days (from the morning of Day 7 to the morning of Day 30). Groups 3 and 4 were fed age-appropriate, non-medicated complete feed from Day 0 to Day 7 and from Day 30 until the end of the study on Day 35. Birds in Groups 1 and 2 received age-appropriate, non-medicated complete feed from Day 0 until the end of the study on Day 35.

(6) Measurements and Observations: From Day 0 to the end of the study on Day 35, the birds and facilities were observed twice daily, with at least four hours between observations. The birds were observed for morbidity, mortality, and other abnormal clinical signs. Animal and feed weights per pen were obtained on Days 7, 14, 21, 30, and 35.

The primary effectiveness variable was the proportion of mortality caused by necrotic enteritis associated with *C. perfringens* from Day 14 to Day 28 (i.e., through 21 days of treatment administration). In order to support an indication for prevention of mortality, the following conditions were required to be met:

- Statistically significant difference in mortality between treated and untreated groups ($p < 0.05$).

- Improvement in mortality between treated and untreated groups that is clinically relevant.
- 80% survival rate for the treated group.

During the study, birds that died or were euthanized for humane reasons were tagged with a unique identification number and necropsied to determine the presumptive cause of death. Birds evaluated prior to the clostridial challenge were not included in the mortality calculations and were considered as normal illness and death associated with poultry production. Necropsied birds were considered a study mortality caused by necrotic enteritis associated with *C. perfringens* if the death occurred after challenge on Day 14, the animal had a gross necropsy diagnosis of necrotic enteritis, and the necrotic enteritis score was ≥ 1. The entire small intestine was scored for necrotic enteritis using the following scale:

0 = Normal, no evidence of gross lesions

1 = Thin-walled, friable small intestines

2 = Focal necrosis and/or ulceration

3 = Multi-focal coalescing areas (large patches) of necrosis

4 = Severe extensive necrosis (typically seen in birds that have died from necrotic enteritis)

In addition, all necropsied birds were scored for coccidial lesions using the system described by Johnson and Reid (*Experimental Parasitology* 1970; 28: 30-38). Both clostridial and coccidial lesions were scored by the same veterinarian.

Secondary variables included intestinal lesion scores, ileal *C. perfringens* counts, growth rate, feed intake, and feed conversion. In addition to the necropsies performed on birds that were moribund or found dead, three randomly selected birds per pen were euthanized on Day 17 and Day 21, necropsied, and evaluated for intestinal lesions. *C. perfringens* counts were obtained from one randomly selected bird per pen euthanized on Day 14 for ileal content sampling and from two of the three birds selected for necropsy on Day 21.

d. Statistical Analysis: The study was designed as a complete randomized block design, consisting of ten blocks, each containing one pen from each of four treatment groups (40 total pens, grouped in 10 blocks). The experimental unit was the pen. Statistical evaluations were made using a two-sided test at alpha = 0.05. For the primary variable, proportion of mortality, a general linear mixed model was evaluated (Mixed procedure in SAS® from the SAS Institute, Cary, NC). Treatment was included in the statistical model as a fixed effect, while block was a random effect. Least Squares Means tests were used to make comparisons between treatment groups and 95% confidence intervals were constructed for differences between pairs of means. Pen average lesion score, assessed on Days 17 and 21, was analyzed as a repeated measures mixed model with

treatment, time, and treatment by time interaction as fixed effects and block as a random effect.

The following comparisons were made:

(1) Group 2 versus Group 1 (adequacy of the clostridial challenge)

(2) Group 2 versus Group 3 and Group 4 separately (effectiveness of avilamycin in preventing mortality)

e. Results: Group 2 had significantly different and higher mortality and lesion scores compared to Group 1, confirming the adequacy of the challenge. Day 14 to Day 28 least squares means mortality caused by necrotic enteritis was significantly different ($p < 0.0001$) in Group 3 (7.5%) compared to Group 2 (30.7%). Least squares means mortality was also significantly different ($p < 0.0001$) in Group 4 (3.0%) compared to Group 2 (30.7%), but was not significantly different from Group 3.

f. Adverse Reactions: No adverse reactions associated with feeding avilamycin were observed during the study. The causes for non-necrotic enteritis mortality or removal were similar among all treatment groups.

g. Conclusions: This study demonstrated that avilamycin fed at an inclusion rate of 15 ppm for 21 days prevented mortality due to necrotic enteritis in broiler chickens challenged with *C. perfringens*.

2. Induced Infection Challenge Model Study

a. Title: "Clinical Study (GCP): Efficacy of Avilamycin to Prevent or Reduce Mortality Related to *Clostridium perfringens* (Necrotic Enteritis) in Broiler Chickens". Elanco Study Number T4EUS120001. April 2013 to May 2013.

b. Study Location: Wellington, Colorado.

c. Study Design:

(1) Objective: To demonstrate the effectiveness of avilamycin Type A medicated article for prevention of mortality caused by necrotic enteritis associated with *Clostridium perfringens* in broiler chickens. The study was conducted according to the VICH Good Clinical Practice (GCP) Guideline (VICH GL9; FDA Guidance For Industry #85).

(2) Test Animals: The study enrolled 1,200 day-of-hatch, male and female Cobb 500 strain broiler chicks. The birds were from the same hatch group and breeder flock. The chicks were randomized to study pens on the day of arrival from the hatchery (Day 0). Each pen contained 40 chicks. The chicks were managed, housed, fed, and watered according to standard, indoor poultry management practices. The chicks were feed two consecutive commercial stage-specific diets: starter feed from hatch to 21 days of age, and then grower feed from 21 days of age to 35 days of age. Birds were uniquely identified on Day 7 (initiation of treatment) via two individual tags containing the same unique number per bird.

(3) Treatment Groups: The study consisted of three treatment groups as provided in Table II.2.

Table II.2. Avilamycin Treatment Groups

Treatment Group	Clostridial Challenge	Treatment Regimen	Number Treated
Group 1	No	0 ppm avilamycin, in feed for 21 days	400 (200 males, 200 females)
Group 2	Yes	0 ppm avilamycin, in feed for 21 days	400 (200 males, 200 females)
Group 3	Yes	15 ppm avilamycin, in feed for 21 days	400 (200 males, 200 females)

(4) Infection Challenge Administration: On Day 14, Groups 2 and 3 were challenged with a *C. perfringens* isolate that induces representative disease in challenged chickens. The isolate was initially collected in July 2007 from a broiler chicken that died during a spontaneous necrotic enteritis outbreak in North America. Following a 6 to 8 hour period of feed withdrawal, the *C. perfringens* challenge was administered in non-medicated starter feed for approximately 16 hours.

(5) Drug Administration: The test article was avilamycin, which was administered via a complete Type C medicated feed. The Type C medicated feed was made from a 10% avilamycin Type A medicated article in the final marketed formulation. Medicated complete feed was provided to Group 3 (15 ppm) for 21 days (from the morning of Day 7 to the morning of Day 28). Group 3 was fed age-appropriate, non-medicated complete feed from Day 0 to Day 7 and from Day 28 until the end of the study on Day 35. Birds in Groups 1 and 2 received age-appropriate, non-medicated complete feed from Day 0 until the end of the study on Day 35.

(6) Measurements and Observations: From Day 0 to the end of the study on Day 35, the birds and facilities were observed twice daily, with at least four hours between observations. The birds were observed for morbidity, mortality, and other abnormal clinical signs. Animal and feed weights per pen were obtained on Days 7, 14, 21, 28, and 35.

The primary effectiveness variable was the proportion of mortality caused by necrotic enteritis associated with *C. perfringens* from Day 14 to Day 28. In order to support an indication for prevention of mortality, the following conditions were required to be met:

- Statistically significant difference in mortality between treated and untreated groups ($p < 0.05$).
- Improvement in mortality between treated and untreated groups that is clinically relevant.
- 80% survival rate for the treated group.

During the study, birds that died or were euthanized for humane reasons were necropsied to determine the presumptive cause of death. Birds evaluated prior to the clostridial challenge were not included in the mortality calculations and were considered as normal illness and death associated with poultry production. Necropsied birds were considered a study mortality caused by *C. perfringens* if the death occurred after challenge on Day 14, the animal had a gross necropsy diagnosis of necrotic enteritis, and the necrotic enteritis score was ≥ 1. The entire small intestine was scored for necrotic enteritis using the following scale:

0 = Normal, no evidence of gross lesions

1 = Thin-walled, friable small intestines

2 = Focal necrosis and/or ulceration

3 = Multi-focal coalescing areas (large patches) of necrosis

4 = Severe extensive necrosis (typically seen in birds that have died from necrotic enteritis)

Coccidial lesions were scored using the system described by Johnson and Reid (*Experimental Parasitology* 1970; 28: 30-38). Both clostridial and coccidial lesions were scored by the same veterinarian.

Secondary variables included intestinal lesion scores, average daily gain, average daily feed intake, growth efficiency, and feed efficiency.

d. Statistical Analysis: A randomized complete block design was used, and each block contained two pens from each of three treatment groups (30 total pens, grouped in 5 blocks). The experimental unit was the pen. The statistical analysis was conducted using a generalized linear mixed model (the GLIMMIX procedure in SAS®, SAS Institute, Cary NC, version 9.3). A binomial distribution was assumed and a logit link used. For any variable that had a significant treatment effect ($p < 0.05$), a Least Squares Means test was used to compare effects between treatment groups.

The following comparisons were made:

(1) Group 2 versus Group 1 (adequacy of the clostridial challenge)

(2) Group 2 versus Group 3 (effectiveness of avilamycin in preventing mortality)

Because of a lack of variability in the untreated, unchallenged group (Group 1), mortality was also analyzed using the pen percent mortality in an analysis of variance (ANOVA) model with random effects for block and replicate within block. For the same reason, necrotic enteritis lesions scores were also analyzed using an ANOVA model with random effects for block and replicate within block.

e. Results: Group 2 had significantly different and higher mortality and lesion scores compared to Group 1, confirming the adequacy of the challenge. Day 14 to Day 28 least squares means mortality caused by necrotic enteritis was significantly different ($p = 0.0008$) in Group 3 (0.5%) compared to Group 2 (7.0%).

f. Adverse Reactions: No adverse reactions attributable to avilamycin were observed during the study.

g. Conclusions: This study demonstrated that avilamycin fed at an inclusion rate of 15 ppm for 21 days prevented mortality due to necrotic enteritis in broiler chickens challenged with *C. perfringens*.

III. TARGET ANIMAL SAFETY

A margin of safety study was conducted that evaluated drug administration for 2X the labeled duration of use. Because the average life span for broiler chickens is approximately 42 days, a longer study duration was not required for this approval.

A. Margin of Safety Study

1. Title: "Non-Clinical Laboratory Study (GLP): Evaluation of the Margin of Safety of Avilamycin in Broilers." Study Number T4EUS110010, April 2013 to April 2014.

2. Study Location: Tulare, California.

3. Study Design:

   a. Objective: The objective was to demonstrate an adequate margin of safety by feeding avilamycin at 0X (negative control), 1X, 3X, and 5X the maximal inclusion rate (45 ppm) to broiler chickens for 42 to 44 days. This study was conducted in accordance with the Good Laboratory Practice Regulations (GLPs; 21 CFR 58).

   b. Study Animals: The test animals were healthy, intact female and male commercial day-of-hatch chicks (commercial broiler chicken (*Gallus gallus domesticus*), strain Cobb X Cobb).

   c. Treatment Groups: The study was a complete randomized block design, with 400 animals (200 males and 200 females) randomized to each of four treatment groups. There were eight blocks each containing four contiguous pens, and each pen in the block contained one of each of the four treatment groups. Each pen contained 50 birds, comprising 25 males and 25 females. The animals were assigned to treatment groups as shown in Table III.1. Each block of four pens was randomized to a necropsy day (Day 42, Day 43, or Day 44) to allow sufficient time for necropsy each day. Blocks were arranged in the facility to provide similar environmental conditions for all pens within a block.

Table III.1. Summary of Treatment Groups.

Group	Treatment Inclusion Rate	Number of Animals
Negative Control	0 ppm avilamycin	400 (200 males and 200 females)
1X	45 ppm avilamycin	400 (200 males and 200 females)
3X	135 ppm avilamycin	400 (200 males and 200 females)
5X	225 ppm avilamycin	400 (200 males and 200 females)

d. Drug Administration: The test article was avilamycin Type A medicated article (100 g/kg, as the final formulation), administered orally as a Type C medicated feed. The control article was unmedicated feed.

Treatments were administered orally in poultry feed appropriate for the animal's age and stage of production. The feed contained either 0 ppm avilamycin (negative control), 45 ppm avilamycin, 135 ppm avilamycin, or 225 ppm avilamycin, and was provided *ad libitum* for 42, 43, or 44 days. Feed and water were available to each pen to provide free-choice consumption.

e. Measurements and Observations: The following parameters were measured and/or observed during the study period: clinical observations (including general health observations, feed and water consumption, and body weight), clinical pathology, and post-mortem (gross and microscopic) examination.

Birds (by pen), feed, and water were weighed on Days 0, 21, and 42. Enrolled animals were observed twice daily for general health status throughout the study. On Days 42, 43, and 44, 2 male and 2 female randomly selected birds from each pen were observed once, had blood collected for analysis, were euthanized, and had a necropsy performed. A gross necropsy was performed on all mortalities that occurred during the study to determine the cause of death.

Clinical pathology analysis consisted of hematology and clinical chemistry. A masked veterinary pathologist performed the gross necropsies. Organ weights were obtained for the brain, heart, liver, and kidneys. Heart, liver, and kidney weights were then compared to brain weights. Samples from normal tissues and from gross lesions were collected for histopathology.

4. Statistical Analysis:

For continuous outcomes measured only once during the study, ANOVA (the MIXED procedure in SAS, SAS Institute, Cary NC; STAT version 12.1) was used to evaluate a model containing treatment as the only fixed effect. The main effect of treatment was evaluated at alpha = 0.10. If this term was significant, pair-wise comparisons of each treatment group against placebo by use of linear contrasts at an unadjusted alpha = 0.10 were performed.

Organ weight (liver, heart, and kidney) to brain ratios and blood values were statistically analyzed by ANOVA (the MIXED procedure). The statistical model included treatment, sex, and the treatment by sex interaction as fixed effects. Pen and animal nested in pen were included in the model as random effects.

Continuous variables measured at multiple times during the study (pen body weight, water consumption, and feed intake) were analyzed by repeated measures analysis of variance, with treatment, day, and the treatment by day interaction as fixed effects, and pen identified as the subject in the repeated statement (the MIXED procedure in SAS, MANOVA).

5. Results:

   a. Clinical Observations and Unscheduled Necropsies: There were 23 early deaths (animals that died or were euthanized prior to completion of the study) occurring across all treatment groups, including controls. The causes of death or euthanasia were attributed variously to inflammation (lung and/or air sac, conjunctiva, femur, brain, or left hock), hemorrhage (epicardium or ileum), bone fracture (femur), or keratoconjunctivitis. The clinical observation and unscheduled necropsy findings were considered typical for chickens of this age and stage of production and were not considered drug-related.

   b. Body Weights: No adverse drug-related changes were observed.

   c. Food and Water Consumption: No adverse drug-related changes were observed.

   d. Hematology: Animals dosed at 225 ppm had significantly different and lower percent basophils than those in the 0 ppm dose group. This finding was not considered clinically relevant because absolute basophil values, the more accurate and clinically relevant measure of basophil quantity, were not found to be significantly different.

   e. Clinical Chemistry: The treatment by sex interaction was statistically significant for globulin, glucose, and uric acid. Within gender, treatment effects were then evaluated for globulin, glucose, and uric acid. Significant differences between the control group and one or more treated groups were observed for globulin, glucose, and uric acid. No dose-related trends were found for any of the serum chemistry parameters, there were no clinical or post-mortem findings associated with the significant differences, and the differences between the treated and control groups were very small. Therefore, these findings were considered either incidental or not clinically relevant.

   f. Post-mortem (gross and microscopic) examination: Post-mortem gross and microscopic changes were distributed across all groups, were sporadic in occurrence, and were attributed to spontaneous changes commonly observed in chickens of this age and stage of production. No significant differences were detected in relative organ weights. Therefore, it was concluded that there were no post-mortem findings attributable to avilamycin.

6. Conclusions: This study demonstrated that INTEPRITY (avilamycin Type A medicated article) is safe for use in broiler chickens when administered as a Type C medicated feed containing up to 45 ppm avilamycin for 21 consecutive days.

IV. HUMAN FOOD SAFETY

A. Antimicrobial Resistance

The Agency evaluated microbial food safety information and data for avilamycin. Avilamycin is proposed for use in Type C medicated feeds at a dose range of 15 to 45 ppm in feed for 21 days, "for the prevention of mortality caused by necrotic enteritis associated with *Clostridium perfringens* in broiler chickens."

The microbial food safety assessment submitted for Agency review included a release assessment to describe the probability that avilamycin and its use at 15 to 45 ppm for 21 days in Type C medicated feeds will result in emergence or selection of antimicrobial-resistant bacteria or antimicrobial resistance determinants in bacteria of public health concern in or on treated chickens under proposed conditions of use; an exposure assessment to describe the likelihood of human exposure to antimicrobial-resistant bacteria or antimicrobial resistance determinants through consumption of edible food products from avilamycin-treated chickens; and a consequence assessment to describe potential human health consequences arising from exposure to defined antimicrobial-resistant bacteria or antimicrobial resistance determinants by considering the human medical importance of orthosomycins used in the treatment of human infectious diseases.

The microbial food safety assessment included information on avilamycin, specifically its spectrum of antibacterial activity, mechanism(s) of avilamycin resistance, and impact on the development or selection of antimicrobial resistance among gram positive foodborne pathogens of concern (*Enterococcus*, methicillin-resistant *Staphylococcus aureus*, and *Clostridium difficile*) as a result of the use of avilamycin in chickens. Gram negative organisms of human health importance such as *Campylobacter*, *Escherichia coli*, and *Salmonella* were not considered a hazard in this risk assessment, as they are intrinsically resistant to avilamycin. In addition, because orthosomycins are not approved for use in human medicine, nor have there been any reports of a human reservoir of avilamycin resistance, there was no data to fully assess the potential impact on human health from the subsequent emergence of antimicrobial resistance as a result of the use of avilamycin in the broiler chicken environment.

Based upon the Agency's evaluation of the information submitted by the firm, and in consideration of the spectrum of activity of avilamycin, including the potential for avilamycin to select for emergence of antimicrobial-resistant bacteria in or on treated chickens, including the prevalence of *Enterococcus* in food products derived from chickens, and taking into considerations the following labeled conditions of use for avilamycin in chicken feed:

- Avilamycin is indicated for, "the prevention of mortality caused by necrotic enteritis associated with *Clostridium perfringens* in broiler chickens",
- Avilamycin will be available as a VFD, and therefore will be administered under veterinary oversight,

- Avilamycin will be administered for 21 days,
- Avilamycin will be administered to chickens beginning at 10 days of age or less,

the Agency concluded that use of avilamycin in chickens will not result in a significant risk of development of avilamycin resistance among foodborne *Enterococcus* originating from treated chickens. The overall risk estimation associated with the use of avilamycin in feed for chickens under the proposed conditions of use is medium, based on individual rankings of medium for the release assessment, high for the exposure assessment, and low for the consequence assessment. The latter ranking of low for the consequence assessment is based on the current lack of use of avilamycin or related analogs in human medicine. The use of avilamycin in feed for chickens, with the risk mitigations listed above, should assure the safe use of avilamycin, and in a manner that would mitigate resistance emergence or selection associated with any adverse impact on human health.

Decision Statement

The Agency's evaluation of information to address microbial food safety risks associated with the proposed use of avilamycin resulted in an overall risk estimation of medium; therefore, administration of avilamycin under the supervision of a veterinarian, limited to a single 21-day treatment in broiler chickens beginning at 10 days of age or less, supports the safe use of avilamycin in broiler chickens and helps to ensure that risks to public health from avilamycin-resistant *Enterococcus* originating from treated broiler chickens have been mitigated.

B. Impact of Residues on Human Intestinal Flora

CVM did not require additional information for the impact of residues on human intestinal flora for this supplemental approval. The FOI Summary for the original approval of NADA 141-438 dated May 8, 2015, contains a summary of all information used to assess the impact of residues on human intestinal flora.

C. Toxicology

Reassessment of the toxicological acceptable daily intake (ADI) was not needed for this approval. The FOI Summary for the original approval of NADA 141-438, dated May 8, 2015, contains a summary of all toxicology studies and information.

D. Establishment of the Final ADI

The final ADI is the toxicological ADI of 1.11 mg/kg body weight (bw)/day or 1110 µg/kg bw/day derived from the 104-week oral carcinogenicity rat study. The codified ADI is listed under 21 CFR 556.68.

E. Safe Concentrations for Total Residues in Edible Tissues

The safe concentrations of total residues of avilamycin in each edible tissue of broiler chickens are 220 ppm for muscle, 660 ppm for liver, 1320 ppm for kidney, and 1320 ppm for fat.

F. Residue Chemistry

1. Summary of Residue Chemistry Studies

a. Total Residue and Metabolism Studies

(1) ^{14}C Avilamycin Balance-Excretion Study in Chickens Study No. ABC-0230

This study was conducted with adherence to appropriate FDA and OECD Good Laboratory Practices (GLPs).

Study Dates: May 6, 1983-August 15, 1983

Study Location: Greenfield, IN

Four chickens (two males and two females), approximately 6-8 weeks of age, were fed unlabeled avilamycin *ad libitum* at 20 ppm for seven days. After seven days administration to approximate steady state conditions, animals received a one-time oral dose of 4 mg of ^{14}C avilamycin in a gelatin capsule. After dosing with ^{14}C avilamycin, animals were fed unmedicated AN22CK ration *ad libitum* for the duration of the experiment. Excreta were collected at 24-hour intervals for 13 days. Radioactivity was measured by combustion followed by liquid scintillation counting.

Table IV.1. Cumulative radioactivity excreted in the feces and measured in chickens dosed orally with avilamycin.

Collection Period (day)	Feces
1	63.71
2	80.22
3	85.38
4	88.09
5	89.34
6	89.76
7	90.89
8	91.32
9	91.78
10	92.17
11	92.38
12	92.50
13	92.65
Mean ± Std Dev	87.7 ± 8.0

The majority of the radioactivity was recovered in the feces (~88%) and was excreted in the first four days, with over 60% coming during the first 24 hours.

(2) ^{14}C Avilamycin Steady-State Tissue Residue Study in Broilers Study No. ABC-0329

This study was conducted with adherence to appropriate FDA GLPs (21 CFR 58).

Study Dates: April 30, 1996-February 28, 1997

Study Location: Greenfield, IN

Eighteen broiler chickens (six males and six females for dosing and three males and three females as controls), approximately 40 days old, were assigned randomly to one of three treatment groups (4 animals/group) and fed a medicated ration *ad libitum* containing 14.16 mg of ^{14}C avilamycin for either four, seven, or ten days. Tissue samples from the muscle, liver, abdominal fat, kidney, and skin with subcutaneous fat were collected at the end of each dosing period. Radioactivity was measured by combustion followed by liquid scintillation counting.

Table IV.2. Mean (± Standard Deviation) Total Radiolabeled Residues measured as mg/kg equivalents avilamycin in broiler chicken tissues.

Dosing Interval (Days)	TRR Muscle	TRR Liver	TRR Skin	TRR Fat	TRR Kidney
4	0.01	0.026 ± 0.01	0.020 ± 0.01	0.088 ± 0.15	<LOD
7	<LOD	0.039 ± 0.03	0.013 ± 0.002	0.028 ± 0.004	<LOD
10	<LOD	0.022 ± 0.01	0.023 ± 0.02	0.031 ± 0.01	<LOD

LOD: 0.008 ppm (muscle); 0.012 ppm (liver); 0.024 ppm (kidney); 0.009 ppm (skin and fat)

Reliable detection and quantitation was demonstrated only for 0.025 ppm. Only one muscle sample (a 4-day sample) with measurable residues at 0.01 ppm was found. After 10 days of dosing, the mean total residues in liver, skin, and fat were 0.022, 0.023, and 0.031 ppm, respectively. Steady state was attained in liver and skin within 4 days and in fat within 4 to 7 days after initiation of dosing. Kidney samples contained no detectable residues at any timepoint.

(3) Total Residues in Edible Tissues and Eggs of Highline W-36 Hens Dosed with ^{14}C Avilamycin in Feed Study No. T4E969602

This study was conducted with adherence to appropriate FDA and OECD GLP standards.

Study Dates: November 1, 1985-January 15, 1986

Study Location: Greenfield, IN

Twenty-four Highline W-36 laying hens, weighing 1.1 to 1.7 kg, were fed a medicated ration *ad libitum* containing 30 ppm of ^{14}C avilamycin for 14 days. Eggs were collected daily throughout the study. Eggs from study days 5, 10, 12 and 14 were separated into yolk and albumin and analyzed for total residues. Tissue samples from thigh and breast muscle, abdominal fat, skin with attached subcutaneous fat, liver, and kidneys were collected on the morning of the 15th day. Radioactivity was measured by combustion followed by liquid scintillation counting.

Table IV.3. Mean (± Standard Deviation) Total Radiolabeled Residues (TRR) measured as mg/kg equivalents avilamycin in chicken tissues.

TRR Liver	TRR Kidney	TRR Muscle	TRR Skin/Fat	TRR Fat
0.08 ± 0.05	0.07 ± 0.02	<LOD	<LOD	0.03 ± 0.01

LOD: 0.04 ppm (muscle); not stated for liver, kidney, skin/fat or fat

Liver and kidney contained the highest amount of avilamycin residues (0.08 and 0.07 ppm, respectively). There were no detectable residues found in muscle and skin/fat.

Table IV.4. Mean (± Standard Deviation) Total Radiolabeled Residues measured as mg/kg equivalents avilamycin in yolk.

Day 1	Day 5	Day 10	Day 12	Day 14
<LOD	0.182 ± 0.04	0.199 ± 0.06	0.214 ± 0.07	0.214 ± 0.06

LOD = 0.13 ppm

Total radiolabeled residues in albumin were not detected (LOD = 0.07 ppm) at any time point. Total residues in yolk averaged 0.214 ppm at 14 days. Day 12 and day 14 yolk residues were not statistically different ($p = 0.34$), thus, steady state was reached by day 12.

b. Comparative Metabolism Study

Because avilamycin residues in chicken tissues are low, a comparative metabolism study was not required for this approval.

c. Study to Establish Withdrawal Period and/or Milk Discard Time

(1) Tissue Residue Depletion Study

Non-Clinical Laboratory Study (GLP): Avilamycin Residue Decline Study in Broiler Chickens Study No. ABC-50406

This study was conducted with adherence to FDA GLPs.

Study Dates: May 12, 2006-December 22, 2006

In-Life Testing Location: Auxvasse, MO

Analytical Laboratory Location: Columbia, MO

Fifty broiler chickens (15 males and 15 females plus 10 males and 10 females as controls) weighing 339 to 541 g, were assigned randomly to one of four treatment groups and fed *ad libitum* a commercial diet containing avilamycin at 150 ppm for 21 days. Tissue samples from the liver, kidney, muscle, and skin with adhering fat (fat/skin) were collected at the end of the 21-day exposure period at withdrawal intervals of 0, 6, and 24 hours (n = 6/group; 3 males and 3 females). Avilamycin and/or its metabolites were determined in chicken tissues using an LC-MS/MS method.

Residues were only detected in liver tissue and declined by more than half in 6 hours. After 24 hours, residues were below or near the limit of quantitation. No residues were detected in muscle or fat/skin at any time. Residues were detected, but not quantifiable, in kidney at 0 and 6 hours withdrawal, and were not detected at 24 hours.

Table IV.5. Final Concentrations of DIA/Avilamycin (ppb)

Withdrawal Time (hours)	Liver	Fat/Skin	Muscle	Kidney
Control	< LOD	< LOD	< LOD	< LOD
0	66.6 ± 31.7	< LOQ	< LOD	< LOQ
6	29.8	< LOD	< LOD	< LOD
24	< LOQ	< LOD	< LOD	< LOD

LOQ = 28 ppb
LOD: 3 ppb in liver; 5 ppb in skin/fat; 4.4 ppb in muscle; 4.9 ppb in kidney

2. Target Tissue and Marker Residue

   Neither a target tissue nor marker residue is assigned.

3. Tolerance

   A tolerance for avilamycin is not needed.

4. Withdrawal Period

   Tissue residue data from Study ABC-0329, T4E969602, and ABC-50406 support a zero-day withdrawal period for avilamycin.

G. Analytical Method for Residues

1. Description of Analytical Method

   Because a tolerance has not been assigned, a validated analytical method is not necessary.

V. USER SAFETY

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to INTEPRITY:

Avilamycin may be irritating to the eyes and may cause allergic reactions in those hypersensitive to avilamycin. Avoid inhalation, oral exposure, and direct contact with skin or eyes. Operators mixing and handling INTEPRITY should use protective clothing, impervious gloves, goggles, and an approved dust mask. Wash hands thoroughly with soap and water after handling. If accidental eye contact occurs, immediately rinse thoroughly with water and seek medical attention. If wearing contact lenses, rinse the eyes first, then remove contact lenses and continue to rinse the eyes thoroughly and seek medical attention. If accidental skin contact occurs, wash all exposed areas of skin thoroughly with soap and water, and seek medical attention if irritation develops. If accidental inhalation occurs, seek medical attention if breathing difficulty occurs. Not for human consumption. If accidental ingestion occurs, call a physician or poison control center. Do not induce vomiting. Keep out of reach of children. The Safety Data Sheet contains more detailed occupational safety information. To report adverse effects in users, to obtain more information, or to obtain a Safety Data Sheet, call 1-800-428-4441.

VI. AGENCY CONCLUSIONS

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR part 514. The data demonstrate that INTEPRITY, when used according to the label, is safe and effective for the prevention of mortality caused by necrotic enteritis associated with *C. perfringens* in broiler chickens. Additionally, data demonstrate that residues in food products derived from broiler chickens treated with INTEPRITY will not represent a public health concern when the product is used according to the label. Prevention of mortality caused by necrotic enteritis associated with *C. perfringens* in broiler chickens is consistent with accepted veterinary practice because an active outbreak of necrotic enteritis can quickly cause significant mortality and no reasonable alternatives to the use of antimicrobials exist to manage necrotic enteritis in certain flocks. As reflected in the labeling, this use is appropriately targeted to broiler chickens that are at risk of developing necrotic enteritis associated with *C. perfringens*. Further, this VFD drug is limited to use by or on the order of a licensed veterinarian who, operating in the course of the veterinarian's professional practice and within the context of a veterinarian-client-patient relationship (VCPR), is equipped by training and experience to determine that the animals to be administered the drug are at risk and to assess whether reasonable alternatives for intervention exist.

A. Marketing Status

A valid VFD is required to dispense this drug. Any animal feed bearing or containing this drug will be fed to animals only by or on a lawful veterinary feed directive issued by a licensed veterinarian in the course of their professional practice.

Labeling restricts this drug to use under the professional supervision of a licensed veterinarian. The decision to restrict this drug to use by or upon a lawful VFD issued by a licensed veterinarian was based on the following factors: (a) adequate directions cannot be written to enable lay persons to appropriately and safely use this product and (b) restricting this drug to use by or upon a lawful VFD issued by a licensed veterinarian should help prevent indiscriminate use, which could result in violative tissue residues.

B. Exclusivity

INTEPRITY, as approved in our approval letter, qualifies for THREE years of marketing exclusivity beginning as of the date of our approval letter. This drug qualifies for exclusivity under section 512(c)(2)(F)(ii) of the FD&C Act because the sponsor submitted an original NADA that contains new studies that demonstrate the safety and effectiveness of INTEPRITY.

C. Patent Information:

For current information on patents, see the Animal Drugs @ FDA database or the Green Book on the FDA CVM internet website.